Effect of Leukocyte Antibodies and HLA Matching on the Intravascular Recovery, Survival, and Tissue Localization of 111-Indium Granulocytes

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The effect of leukocyte antibodies detected under different conditions on the fate in vivo of granulocytes was studied using 111-indium-labeled granulocytes. Sera from patients were tested by granulocyte agglutination (GA), granulocyte cytotoxicity (GC), granulocyte immunofluorescence (GIF), lymphocytotoxicity (LC), and antibody-dependent lymphocyte-mediated granulocytotoxicity. Granulocytes from donors to be studied were labeled with 111-indium and injected. Then the intravascular recovery and survival of tissue localization was determined in 93 studies. Antibodies detected by granulocyte agglutination were associated with a significant reduction in recovery (6.7% v 30.8% in controls; P < .001) and t1/2 (0.3 hours v 5.6 hours in controls; P = .002). When all possible combinations of serum reactivity were considered, reactivity in the GA plus GIF assays had the best correlation with decreased recovery (R² = .49; P < .001) and t1/2 (R² = .73; P < .001). When the relationship between the strength of antibody reactivity and the recovery and t1/2 were analyzed, the best relationship was between the combination of LC and GIF with recovery (R² = .62; P = .001). Because of the general availability of the HLA (LC) testing, the role of LC reactivity was investigated in other ways. There was a strong relationship between sera highly reactive by LC and those reactive by GIF. These highly reactive sera were also associated with reduced recovery and t1/2. The influence of specific HLA antigen mismatches was also studied. When donor and recipient were mismatched for the HLA-A2, B8, or BW44 antigens, there was a significant reduction in either recovery, t1/2, or both. Tissue localization was studied by body scans in patients with and without known sites of inflammation. Antibodies detected by a combination of GA and GIF caused abnormal pulmonary sequestration of granulocytes (three cases) and failure of granulocytes to localize at known sites of inflammation (three cases). HLA (LC) antibodies did not alter tissue localization despite the presence of the corresponding HLA antigens on granulocytes. It appears that GA, GIF, or a combination of these tests is the most effective predictor of altered in vivo fate of granulocytes. However, sera highly reactive by LC and GIF probably define a group of highly immunized patients in whom granulocyte recovery and t1/2 are also reduced. Mismatching for certain HLA antigens is also associated with reduced granulocyte recovery and survival. At present, GA, with or without the immunofluorescence assay, is the most effective predictor of altered in vivo fate of granulocyte activity. LC, if interpreted in a specific manner, may also define a group of highly immunized patients in whom in vivo granulocyte activity is altered.

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EUKOCYTE ALLOANTIBODIES play a role in febrile and pulmonary transfusion reactions, autoimmune neonatal neutropenia (INN), response to granulocyte transfusions, drug-related neutropenia, and autoimmune neutropenia.1 Many methods are available to detect leukocyte antibodies; however, the relationship of these tests to immune destruction of granulocytes, and thus to these different clinical situations, is not established.2 4 Because most of the assays are complex, there have been few studies comparing the value of different antibody methods in detecting clinical problems. For instance, in different reports, autoimmune neutropenia has been associated with antibodies detected by agglutination, cytotoxicity, immunofluorescence, opsonization, antibody-dependent cell-mediated cytotoxicity, and staphylococcal protein A.1.6 Immune neutropenia secondary to drugs may be caused by granulocyte agglutinins, opsonins, or cytotoxins1.7 and secondary to Felty's syndrome, by antibodies detected by antibody-dependent lymphocyte-mediated granulocytotoxicity.4 Transfusion reactions are associated with leukoagglutinins and lymphocytotoxins (HLA antibodies), while antibodies detected by both granulocyte agglutination and immunofluorescence have been found in INN. A poor response to granulocyte transfusions has been attributed to different antibodies, including leukoagglutinins, lymphocytotoxins,8-11 and those detected by immunofluorescence.12-14 Although others14 have reported no effect of granulocyte agglutinins, granulocytotoxins, or lymphocytotoxins on granulocyte intravascular recovery.

Until recently, there has not been a method available to study the in vivo fate of a single injection of a granulocyte suspension so that the effect of antibodies present at the time could be determined. The isotope 111-indium is an efficient granulocyte label, does not elute from the cell, and emits photons in high abundance, making it ideal for external body imaging.15 Thus 111-indium granulocytes can be used to determine both the intravascular kinetics and the extravascular localization of granulocytes.16-17 We have used 111-indium granulocytes to study the effect of leukocyte antibodies detected in five assays on the in vivo fate of granulocytes.

This report is an extension of our previous work.17 Thirty-five new studies were done using all five leukocyte antibody methods, and two additional antibody methods were applied to the 53 studies previously reported.

MATERIALS AND METHODS

This study was approved by the University of Minnesota's Committee of the Use of Human Subjects in Research. All participants gave informed consent. Subjects in this study were either patients who were receiving platelet or granulocyte transfusions or those for whom an indium scan had been requested for diagnostic purposes.
because localized infection was suspected. On the morning of the indium study, blood was obtained from the patients and normal donors. A suspension of donor granulocytes was prepared, labeled with $^{111}$indium, and injected into the intravascular system and organ localization studies were performed during the subsequent 24 hours. A cross-match between the recipient’s serum and donor’s granulocytes and lymphocytes was done using five leukocyte antibody methods. Sometimes it was not possible to do all five crossmatches on the day of the indium study. In those cases, the patient’s serum from that day was stored at $-70{\degree}C$ and used for crossmatching later with granulocytes or lymphocytes freshly obtained from the original donor. The granulocyte donors met all of the criteria of the American Association of Blood Banks for whole blood donation and were hepatitis B, antigen negative. All of the methods used in this study have been reported in detail previously and are summarized here.

**Methods**

**Granulocyte agglutination.** Granulocyte agglutination (GA) was done as previously described using microtiter trays. The results were read after five hours of incubation at $30{\degree}C$. The strength of reactivity was graded as 0, 1, 2, 3, or 4, according to the percentage of granulocytes that agglutinated.

**Lymphocytotoxicity.** Lymphocytotoxicity (LC) was done using the standard National Institutes of Health method. $^9$ The strength of the reaction in the crossmatch was graded as 0, 1, 2, 3, 4, or 5, based on the percentage of stained lymphocytes. Sera that had an incompatible LC crossmatch against donor cells were also screened against a panel of lymphocytes from 60 donors containing 20 HLA-A locus, 31 B locus, and eight C locus antigens. The percentage of the 60 panel cells that reacted was recorded, and the specificity of the HL A antibody reactivity determined by analyzing this pattern of reactivity.

**Granulocytotoxicity.** For granulocytotoxicity (GC), the method of Hasegawa et al $^{10}$ was used, except that blood for preparation of the cell suspension was collected in acid-citrate-dextrose (ACD), granulocytes were isolated using a double-density Ficoll-Hypaque gradient, not papainized, and the sensitization phase of incubation was carried out at 22 $^0$C and 37 $^0$C. The test was considered positive when more than 40% of cells were stained. The strength of reactivity was graded as 0, 1, 2, 4, 6, or 8 in relation to the percentage of stained granulocytes.

**Granulocyte immunofluorescence.** Granulocyte immunofluorescence (GIF) was done essentially as described by Verheugt et al, $^{11}$ except that granulocytes were isolated using the double-density Ficoll-Hypaque gradient and not subjected to hypotonic lysis of red cells.

**Antibody-dependent lymphocyte-mediated granulocytotoxicity.** This was our modification of the method of Logue et al. $^{12}$ Antibody-dependent lymphocyte-mediated granulocytotoxicity (ADLG) has been described in detail and is summarized here. Blood was drawn into heparin, and the mononuclear cells were isolated by a double-density Ficoll-Hypaque gradient and not subjected to hypotonic lysis of red cells. Phagocytic cells were removed by carbonyl iron, and the lymphocytes were isolated, washed, and suspended in McCoy's medium containing 15% fetal bovine serum. Granulocytes were isolated from the double gradient, washed, labeled with 200 $\mu$Ci of sodium $^{51}$chromate, washed again, and resuspended in phosphate-buffered saline. The reaction mixture consisting of 10 $\mu$L of test or control serum and 10 $\mu$L of $^{51}$Cr granulocyte suspension was incubated in U-bottom microtiter plates for 30 minutes at 37 $^0$C in 5% $CO_2$. Then 130 $\mu$L of the lymphocyte suspension was added to give an effector-target cell ratio of 50:1. The mixture was then incubated for four hours at 37 $^0$C, 50 $\mu$L of cell-free supernatant was removed, radioactivity measured, and the percentage of cytotoxicity calculated. Controls included the following: (1) sodium dodecyl sulfate-treated granulocytes to measure the total release of $^{51}$Cr; (2) AB serum from a nontransfused male to measure spontaneous release of $^{51}$Cr in the presence and absence of lymphocytes; (3) O negative control serum; (4) autologous serum from the granulocyte donor; (5) positive control serum (anti-NAl, anti-NBl). Previous studies established a mean of 2.7% radioactivity released (cytotoxicity) in normal subjects. $^{13}$ A percentage of granulocytotoxicity greater than 2 SD above this mean was considered positive (cytotoxicity >10%) if the negative control values were within 2 SD of the normal mean.

**Studies Using $^{111}$Indium Granulocytes**

**Preparation and labeling of granulocytes.** Granulocytes for $^{111}$In labeling were processed, as previously described, with the use of a discontinuous Ficoll-Hypaque gradient from 125 mL ACD whole blood obtained by phlebotomy. The suspension contained an average of $1\times10^8$ granulocytes with less than 1% mononuclear leukocytes and less than 5% red blood cells.

$^{111}$Indium-111-oxine was prepared as previously published. $^{14}$ The granulocyte suspension was incubated with indium-111-oxine at room temperature for 20 minutes with occasional gentle mixing and then infused into the recipient. $^{15}$ The small amount of labeled cell suspension remaining in the vial was used to determine labeling efficiency and the number of cells injected.

**Determination of intravascular recovery and $t_{1/2}$.** Blood samples were obtained from the recipient at approximately ten minutes, 30 minutes, and 1, 2, 3, 4, 5, and 6 hours after injection, and the granulocyte-associated radioactivity was determined. $^{16}$ Calculation of the percentage of injected granulocytes recovered in the circulation and their intravascular half-life ($t_{1/2}$) was based on total cell-associated activity injected and the recipient's blood volume as previously described. $^{17}$

**Body scans.** Approximately 20 hours after injection of the indium-labeled granulocytes, the subjects underwent total body scanning using a Cleon body scanner (Union Carbide, Danbury, Conn). With this technique, indium-labeled granulocytes are normally seen in the liver and spleen. Thus the scans were classified as follows: true negative—radioactivity in liver and spleen and no evidence of clinical inflammation found on follow-up; true positive—radioactivity in liver and spleen and at the site of inflammation present at the time of the scan; false positive—radioactivity in liver, spleen, and other sites in the absence of inflammation; false negative—radioactivity in the liver and spleen, with none at a site of inflammation present at the time of the scan. The presence or absence of inflammation was determined by continued follow-up of the patients and review of their entire clinical course by one of us (J. McCullough, C. Ludvigsen, L. Forstrom), who did not know the results of the scan when making the final decision regarding inflammation.

**Statistical analysis.** Patients were grouped based on the kind of antibody present and the presence or absence of neutropenia. Each group of patients with antibodies was compared with patients matched for the presence or absence of neutropenia but with no antibodies. The mean value for recovery and $t_{1/2}$ for each group was determined, and differences between groups were evaluated by two-sample Student's $t$ test. The degree of the linear relationship between kinetics values (percentage of recovery and $t_{1/2}$) and the strength of individual crossmatch scores was determined using a Pearson Correlation Coefficient test. Multiple regression analysis was used to examine the predictability of the association between combinations of crossmatch results and the kinetics values. The differences in kinetics values among the groups of patients who received HLA-mismatched transfusions were tested by two-sample Student's $t$ test. The relationship between positive and negative
results in the five leukocyte antibody tests was determined using two-by-two contingency tables. The statistical significance was calculated using chi-square with the Yates correction factor. Differences in all tests were considered significant if \( P < .05 \).

**RESULTS**

A total of 93 indium studies (partial results of 58 were previously reported in reference 17) was done in 38 adult and 45 pediatric patients (some patients were studied more than once). The patients had a variety of diseases, including aplastic anemia, acute or chronic leukemia, solid tumors, postoperative suspected localized infection or sepsis, or were post bone marrow transplant. Some patients were receiving platelet or granulocyte transfusions, and some were undergoing an indium scan to detect suspected occult infection. Leukocyte antibody testing and body scans were done in all 93 studies, and granulocyte kinetics data were obtained in 53 studies.

**Intravascular kinetics studies.** In our previous experience in analyzing the present data, neutropenic patients had significantly reduced granulocyte recovery, and the presence of documented infection did not influence the intravascular recovery or \( t/2 \). Therefore, patients in the present study were categorized based on the presence or absence of neutropenia (<1,000 PMN/µL), but not infection. In general, these patients were severely neutropenic. During 18 studies (Figs 1 and 2), the neutrophil count was known to be less than 1,000/µL but was not quantitated further. In the other 30 studies, the neutrophil counts were as follows: 1,000 to 500/µL, three studies; 500 to 200/µL, nine studies; and <200/µL, 18 studies.

In the 53 studies involving intravascular kinetics of indium granulocytes, no antibodies were detected in any of the five assays in 18 studies, nine in neutropenic, and nine in nonneutropenic patients (Figs 1 and 2). The average intravascular recovery and \( t/2 \) in nonneutropenic patients (30.8% and 5.6 hours) were similar to our previous results in nonneutropenic patients without antibodies and in normal subjects who received autologous granulocytes. In neutropenic patients, the average intravascular recovery (10.7%) and \( t/2 \) (5.4 hours) were also similar to our previous results in neutropenic patients. In 18 studies, antibodies were detected by only one of the five methods, and in 17 studies, antibodies were detected by two or more methods (Figs 1 and 2). Compared with patients with no antibodies, there was a significant reduction in granulocyte recovery and \( t/2 \) in the one nonneutropenic patient with only a GA antibody (6.7% v 30.8%; 0.3 hours v 5.6 hours) and in the four nonneutropenic patients who had a combination of GA plus other antibodies (9.7% v 30.8%; 0.3 hours v 5.6 hours). A statistically significant decrease in \( t/2 \), but not recovery, also occurred in neutropenic patients with GA antibodies alone or in combination with others. Because of this demonstrated effect of GA antibodies, those patients were removed from the other groups in the remainder of this analysis.

One nonneutropenic patient had antibodies detected by GIF and other methods. The recovery (16.2%) and the \( t/2 \) (1.4 hours) in this patient were significantly less than in comparable patients with no antibodies. The average recoveries in all other groups were not significantly less than in patients without antibodies. It should be emphasized that in the nine patients who had LC (HLA) antibodies alone or in combination with other antibodies, there was no significant reduction in the intravascular recovery or \( t/2 \) of incompatible granulocytes when there was no accompanying granulocyte agglutinating activity (Figs 1 and 2). In four groups of neutropenic patients, either the recovery or \( t/2 \) values were significantly higher than in the control patients with no
antibodies (Figs 1 and 2). In several of these situations, the data involved only one study. Six of the 14 patients involved had acute myelocytic leukemia and seven were infected. However, none had overwhelming infections or sepsis. No common features were apparent in these patients. Thus we believe that these statistically significant differences are probably due to the difficulty in standardizing all variables in these clinically ill patients and do not represent a causal relationship.

In order to analyze the relationship between multiple antibodies and the recovery or $t'/2$ of indium granulocytes, a multiple regression analysis was done comparing all possible combinations of antibodies with the recovery and $t'/2$. The presence of both GA and GIF antibodies had the best correlation with decreased recovery ($R^2 = .49; P < .001$); however, most of this effect was due to the GIF testing (GIF, $R^2 = .47$; GA, $R^2 = .28$). The combination of GA and GIF also had the best correlation with survival ($R^2 = .73; P < .001$); however, the GA and GIF made similar contributions (GA, $R^2 = .61$; GIF, $R^2 = .58$). In neutropenic patients, no combination of antibodies gave a correlation with recovery better than $R^2 = .26$.

The relationship between the strength of reactivity of the antibody and the recovery or $t'/2$ was analyzed. The Pearson Correlation Coefficient was used to test individual variables, that is, sera reactive in only one of the assays. Because the Pearson Correlation Coefficient requires a sample size of at least three, only GC testing could be analyzed. There was no significant relationship between the strength of GC reactivity and the percentage of recovery ($P > .34$) or the $t'/2$ ($P > .29$). Multiple regression analysis was used to study sera reactive in more than one assay. In nonneutropenic patients, there was a significant relationship between the strength of reactivity in the combination of GIF and LC assays and the recovery ($R^2 = .62; P < .001$). The strength of reactivity in the GIF alone was significantly related to the $t'/2$ ($R^2 = .60; P < .001$). Similar tests in neutropenic patients did not yield significant relationships possibly because the values for recovery in patients without antibodies were low and many studies would be necessary to show statistically significant differences.

Because of the relationship between the strength of reactivity of the LC and GIF with granulocyte recovery, and because of the wide availability of HLA (LC) testing, the role of HLA was investigated in more detail in several ways. The extent of reactivity of an HLA antibody can be determined not only by the degree of cytotoxicity against the individual donor cells in the crossmatch test, but also by the percentage of different random panel donors whose cells react. Those sera that reacted with 60% of different donors' lymphocytes were arbitrarily designated as highly reactive.

Of 13 sera that reacted with <60% of panel donors, few reacted by GA, GIF, GC, or ADLG (Table 1), and the granulocyte recovery and $t'/2$ were normal in three of four patients studied. Of 15 sera that reacted with >60% of panel donors, 14 (93%) also reacted by GIF. This was consistent with the observation that there was a statistically significant relationship between LC and GIF results ($P < .001$) but not between LC and other methods ($P > .05$). The granulocyte recovery and $t'/2$ were reduced in six of the eight patients studied whose sera were highly reactive by LC. The remaining two sera associated with the normal recovery and $t'/2$ did not contain GA antibodies. However, three of the six sera associated with reduced recovery and $t'/2$ were also GA negative.

The effect of HLA antigen differences between donor and recipient was analyzed in combinations in which the donor's cells contained an antigen lacking in the recipient. The recovery and $t'/2$ for these combinations was compared with similar patients in whom either the HLA types of donor and patient were identical or the donor cells lacked an antigen that the recipient's cells contained.

There were 166 observations in which the patient's serum contained antibodies, but donor cells did not contain an HLA antigen lacking in the recipient (called matched, Table 2). There were 34 observations in which the patient's serum reacted against the donor's cells in at least one of the five assays and the donor cells contained an HLA antigen lacking in the recipient (called mismatched, Table 2). Significant findings involved HLA-A2 and BW44. There was a reduced $t'/2$ in the seven patients who received HLA-A2-incompatible granulocytes and reduced recovery and $t'/2$ in the three patients who received HLA-BW44-incompatible granulocytes.

### Table 1. Relationship of Reactivity of Sera in the LC (HLA) Test to the Reactivity in Other Tests and to Reduced Granulocyte Recovery (R) and Survival ($t'/2$)

<table>
<thead>
<tr>
<th>Panel Donors Reactive (%)</th>
<th>Sera Reactive by (%)</th>
<th>Reduced R and $t'/2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n GA GIF GC ADLG</td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>13 8 23 38 31</td>
<td>1 of 4</td>
</tr>
<tr>
<td>&gt;60</td>
<td>15 33 93 47 20</td>
<td>6 of 8</td>
</tr>
</tbody>
</table>

### Table 2. Influence of Donor-Recipient Mismatching for Selected HLA Antigens on the Recovery and Survival of 111-Indium Granulocytes

<table>
<thead>
<tr>
<th>HLA Antigen</th>
<th>Donor Recipient</th>
<th>Mismatched Donor Recipient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>$t'/2$ (hours)</td>
</tr>
<tr>
<td></td>
<td>Matched</td>
<td>Mismatched</td>
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<td></td>
<td>n %</td>
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Results shown are from 34 studies involving 18 patients whose serum reacted in one or more of the leukocyte antibody assays compared with 166 studies in 20 patients with antibodies in one or more leukocyte antibody assays and matched for selected HLA antigens.

* $P < .05$. From www.bloodjournal.org by guest on November 15, 2017. For personal use only.
cytes. Both the recovery and $t_{1/2}$ in HLA-B8 mismatched were reduced but not statistically significant ($P = .06$). Because of the observed effect of GA antibodies on recovery (Fig 1), the six patients with reactivity in the GA assay were excluded and the data on the remaining 12 studies was reanalyzed. Similar results were obtained; that is, there was a significant reduction in recovery and $t_{1/2}$ with HLA-A2 and BW44 positive cells (data not shown).

Some that react by LC often have broad reactivity and their HLA antigen specificity cannot be determined. Four patients who had HLA antibodies of clear specificity received granulocytes that contained the corresponding antigen; HLA-A2 (two cases) and A24 (two cases) (Table 3). None of the patients had GA reactivity, so that was not a confusing factor. In three patients (1 through 3), two with anti-HLA-A2 and one with anti-HLA-A24, it appears that the recovery of the incompatible donor cells was reduced and the $t_{1/2}$ was shortened, although $P$ values were not calculated because of the small number of studies done.

Tissue localization. Ninety-three indium scans were done, 45 of which were in patients determined to have localized inflammation at the time of the study. No antibody was detected by any of the five methods in 27 of the 93 studies. Thirty-two studies were done in patients who had an antibody detected by only one of the five methods (GA, four; GC, 12; LC, four; GIF, one; and ADLG, 11). (Table 4). Thirty-four other patients had antibodies detectable in more than one assay.

One of the patients with only a GA antibody had a true positive scan. She was a 4-year-old female with acute myelogenous leukemia, esophagitis, and colitis. There was localization of indium-granulocytes in the esophagus and colon. This is the only true positive scan we have seen in the presence of a GA antibody in more than four years of study. The antibody reacted weakly and gave an unusual, loose-appearing agglutination with only a few cells in each agglutinate, which may account for their ability to localize in vivo.

GA antibodies were present with others in 12 studies (Table 4). Six were true negatives and the previously mentioned child had a second positive scan at a time when the serum reacted by GC as well as by GA. Two patients had false negative scans. One had clinically apparent submandibular cellulitis and one, osteomyelitis at a leg amputation stump. A third patient is classified as both false negative and false positive. This 22-year-old female post renal transplant because of juvenile-onset diabetes had an intra-abdominal abscess at which indium granulocytes failed to localize (false negative) but showed pulmonary sequestration of indium granulocytes in the absence of evidence of pulmonary inflammation (false positive). Two additional patients with GA antibodies had abnormal pulmonary sequestration of indium granulocytes, and thus were classified as false positives. One was a 72-year-old man with chronic autoimmune neutropenia (Fig 3), whose serum reacted in the GA, GC, GIF, and ADLG tests. The intravascular recovery of granulocytes was reduced to 4.6% (normal, 10.7%), and their survival was short ($t_{1/2}$, 0.2 hours; normal, 5.4 hours). Severe abnormal bilateral pulmonary uptake of indium granulocytes was apparent throughout the 24 hours postinjection. The other was a 9-year-old female post bone marrow transplantation for acute myelogenous leukemia. Neither patient had evidence of pulmonary inflammation and neither was on any drug that was likely to cause pulmonary toxicity. All of these patients who had false negative or false positive scans had serum reactivity in the GA and GIF assays. Many patients whose serum reacted in one or more other tests had true positive scans and none had false negative or false positive scans (Table 4).

DISCUSSION

In this study, granulocyte agglutinating antibodies alone and in combination with other leukocyte antibodies were
associated with decreased intravascular recovery and survival of incompatible granulocytes. In addition, antibodies detected by GA in combination with GIF were associated with failure of granulocytes to localize at sites of inflammation or with abnormal pulmonary sequestration, or both.

Analysis of the data in this manner did not indicate an important role for LC testing and the HLA system in granulocyte destruction, lack of migration to inflammatory sites, or abnormal pulmonary sequestration. However, because HLA antigens are found on granulocytes and there is widespread use of LC testing, we analyzed donor recipient combinations in which the donor's granulocytes contained HLA antibodies lacking in the recipient. In patients whose serum reacted in one or more of the antibody assays, mismatching for HLA-A2, B8, or BW44 was associated with reduced recovery and survival of injected granulocytes. In four patients it was possible to examine the effect of the specific HLA antibodies anti-A2 and A24. There was a strong suggestion that these antibodies were associated with a reduced recovery and survival of donor granulocytes containing the corresponding antigen. Sera reactive by LC (HLA) against a broad range of panel donors (>60%) also were associated with reduced recovery and $t'/2$. There was a statistically significant association between reactivity of these sera and reactivity by GIF. Thus the combination of LC and GIF may define a group of highly immunized patients in whom the recovery and survival of injected granulocytes are reduced.

Ungerleider et al\textsuperscript{4} found that neither the increment in granulocyte count after transfusion nor transfusion reactions were related to the presence of leukocyte antibodies detected by GC, LC, leukoagglutination, or capillary agglutination. The major shortcoming of their study, however, was the use of the posttransfusion granulocyte count to indicate a successful transfusion in granulocytopenic patients. Because of the effect of many patient variables on granulocyte counts in this range, this is probably not a satisfactory method of evaluating a single injection of granulocytes.

Two studies in dogs have established that prior exposure to blood from the granulocyte donor caused a decreased posttransfusion increment and migration into a skin chamber.\textsuperscript{24,25} In one study,\textsuperscript{24} testing by LC revealed antibodies in only 30% of the immunized dogs, while in the second study,\textsuperscript{25} most immunized dogs had antibodies detected by LC, GC, and agglutination. Thus the optimum serologic tests to detect immunization in this dog model were not revealed from these studies. Subsequently, however, Chow and Epstein,\textsuperscript{15} using a neutropenic dog model, showed that transfusions of granulocytes incompatible by GIF resulted in significantly lower increases in peripheral granulocyte count than in compatible transfusions. There was not a good correlation of granulocyte increment with lymphocytotoxicity or leukoagglutination.

Studies in humans also indicate that antibodies detected by immunofluorescence may have clinical significance. In 25 patients receiving granulocyte transfusions because of gram-negative sepsis, the presence of GIF antibodies was associated with death in five of six and their absence associated with recovery in 12 of 13.\textsuperscript{12} This relationship was not found when clinical outcome was compared with lymphocytotoxicity (HLA) antibody results.

Dutcher et al\textsuperscript{26} reported that indium-labeled granulocytes localized at sites of infection in 20/20 nonimmunized patients but in only 3/14 immunized patients. Alloimmunization also significantly increased pulmonary retention of indium-labeled granulocytes during the 30 minutes after injection.\textsuperscript{27} In both studies, immunization was defined as the presence of HLA (LC) antibody. However, most patients had both lymphocytotoxic and leukoagglutinating antibodies so that the relative role of HLA and granulocyte antibodies could not be established.

Our studies and those of others summarized here do not establish which antigen system(s) is (are) involved in interference with the in vivo fate of granulocytes. GA detects neutrophil specific and some HLA antigens; GIF detects both neutrophil specific and HLA antigens, whereas LC primarly detects HLA antigens. This study indicates that immune destruction of granulocytes can be predicted by the GA test, or even more accurately by the combination of GA and GIF tests. The association between LC and GIF reactivity and the observation of reduced recovery and $t'/2$ when some HLA antigens were mismatched suggests that LC antibodies and the HLA system are involved in granulocyte destruction. However, this is not well predicted by the usual interpretation of the LC assay. It appears that at present, the GA assay, if possible supplemented by the GIF, gives the best prediction of in vivo granulocyte kinetics. Possibly testing sera by LC against a large number of panel cells can also be used to identify highly immunized patients in whom granulocyte recovery and survival will be reduced.
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